1973

Effects of carbachol induced drinking on conditioned saccharin aversions in rats

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by

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A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of
The Requirements for the Degree of
DOCTOR OF PHILOSOPHY

Major: Psychology

Approved

Signature was redacted for privacy.

In Charge of Major Work

Signature was redacted for privacy.

For the Major Department

Signature was redacted for privacy.

For the Graduate College

Iowa State University
Ames, Iowa

1973

TABLE OF CONTENTS

INTRODUCTION

It is possible to activate various behavioral patterns such as eating, drinking, and emotional activity by electrical or chemical stimulation of specific brain sites. It is questionable, however, whether similar motivational patterns underlie the naturally occurring and artificially induced states, and also whether the same neural mechanisms are involved. The present experiment was designed to investigate the relationship between natural thirst and chemically induced thirst with particular emphasis on the motivational properties associated with each condition.

Investigations of central neural mechanisms underlying feeding and drinking behaviors have been conducted using both stereotaxic lesion and stimulation techniques. In many instances artificial stimulation, either electrical or chemical, elicits behavior patterns that are similar to the patterns of food and water seeking behavior of naturally deprived animals. Miller (1957, 1960) has stressed the importance of employing a wide variety of measures in studying consummatory behavior and has shown that electrical stimulation of the hypothalamus in rats does not merely elicit reflex-like gnawing responses, but also initiates the performance of learned food rewarded responses. Nondeprived animals learned to press a bar on a variable interval (VI) schedule of reinforcement to obtain food immediately following the onset of hypothalamic electrical stimulation. Injections of hypertonic saline solution into the lateral ventricles of a cat increased the rate of responding for an intermittent water reward while injections of water

reduced performance.

Andersson, Larsson, and Persson (1960) have also demonstrated that electrical stimulation of the goat hypothalamus elicits drinking and learned water-reinforced responses. When the goat was stimulated but not allowed access to water a displacement reaction (abdominal scratching) occurred. This reaction appeared to be similar to tension or frustration that results from being unable to drink. This displacement reaction offers additional evidence for the elicitation of a strong drinking urge by electrical stimulation. Thus, it appears possible to artificially activate not only isolated consummatory reflexes but also motivational systems which underlie hunger and thirst.

Investigations of the precise anatomical localization and specific chemical coding of the neurons involved in consummatory behavior was initiated by Grossman (1962a,b). Double-walled cannula assemblies which allow repeated stimulation of a neural site with crystalline chemicals were implanted in the lateral hypothalamic area in rats. Placement of an adrenergic substance, norepinephrine, into the lateral hypothalamus induced feeding in sated rats and also increased the food intake of deprived animals. Placement of cholinergic substances, acetylcholine or carbachol, into the same locus elicited drinking in sated rats and also increased the amount drunk by deprived rats. Adrenergic drugs also inhibited drinking whereas cholinergic drugs inhibited eating. In a two bar situation adrenergic stimulation increased the performance on the food rewarded bar while cholinergic stimulation increased performance on the water rewarded bar. Thus, the

motivational properties of chemically induced hunger and thirst appear to be similar to natural hunger and thirst.

Miller, Gottesman and Emery (1964) determined the dose-response curves for liquid injections of carbachol and norepinephrine. An inverted U shaped dose-response curve was obtained indicating that negative results may be obtained when either a too high or too low dose is administered. Carbachol stimulation of the lateral hypothalamus elicited drinking of water instead of a liquid food while stimulation with norepinephrine elicited drinking of liquid food rather than water. High doses of carbachol induced severe motor seizures.

The selective sensitivity of lateral hypothalamic neurons to adrenergic and cholinergic precursors and blockers has also been demonstrated (Grossman, 1962b). The adrenergic precursor dopamine induced eating while dimethylaminoethonal, a cholinergic precursor, elicited drinking. Adrenergic and cholinergic blockers, ethoxybutamoxane and atropine, decreased eating and drinking respectively. The effects of adrenergic and cholinergic stimulation and the effects of the precursors and blockers could be elicited from the identical anatomical locus using the same cannula, indicating that the hunger and thirst systems overlap anatomically but are activated by specific chemical substances.

Stein and Seifter (1962) reported that the drinking that followed cholinergic stimulation resulted from muscarinic rather than nicotinic stimulation of hypothalamic neurons. Administration of muscarine into the cholinergically active site produced the same effects as carbachol while nicotine had little effect. Pretreatment with atropine, a

muscarinic rather than nicotinic blocking agent, abolished the effect of muscarine.

Thirst circuit

Fisher and Coury (1962) subsequently demonstrated that the perifornical region of the lateral hypothalamus stimulated by Grossman (1962a,b) was not the only brain area which was selectively responsive to cholinergic stimulation. Drinking was elicited following carbachol stimulation of the dorsal hippocampus, cingulate cortex, septal area, lateral hypothalamus, and other structures which anatomically form the classic Papez limbic circuit. From this research it appears that the regulation of water intake is not under the unitary control of a single neural "center" but rather, an entire cholinergically coded "circuit" consisting of a complex pattern of alternative and reciprocal pathways may be involved in the mediation of thirst.

The circuit theory of thirst was supported by Levitt and Fisher (1966) who demonstrated that when atropine, a cholinergic blocker, was applied to any positive drinking site, carbachol induced drinking could not be elicited from any other site in the thirst circuit. These results indicate that all components of the limbic thirst circuit must be functional for drinking to occur.

Levitt (1969) investigated the effects of various chemical agents on cholinergically induced thirst when they were applied to the same site as carbachol or to a different site within the circuit. Atropine sulfate and scopolamine hydrobromide, both muscarinic blocking agents, were the most potent blockers of cholinergically induced water intake.

A greater reduction in drinking occurred when the blocking agent was applied to a site not treated with carbachol. This result might be expected since an anticholinergic agent placed in a site treated with carbachol would have to block the action of both the acetylcholine which is normally present and the action of carbachol which was artificially implanted. It would be necessary for a blocker in a different site to block only the naturally occurring acetylcholine in order to interrupt the circuit and prevent drinking.

Levitt and Boley (1970) injected a cholinesterase inhibitor, eserine, into various limbic structures and found a high correlation between those sites from which drinking was elicited by both carbachol and eserine. Since eserine inhibits cholinesterase, drinking would result from a build up of endogenous acetylcholine. When eserine was injected into one positive drinking site and atropine into another, a reduction in eserine-induced drinking occurred. Although carbachol elicited more drinking than eserine, it appears that both drugs operate at identical loci.

Drinking has also been elicited by placement of crystalline carbachol into the anterodorsal hippocampus while cholinergic stimulation of the posteroventral hippocampus has little effect on drinking (Grant and Jarrard, 1968). Application of norepinephrine to both hippocampal areas resulted in increased eating. Thus, the hippocampus receives both cholinergic and adrenergic fibers, and there exists both a neuroanatomical and a neurochemical dissociation between the hunger and thirst systems within the hippocampus.

Fisher and Coury (1962) found that the sites that produced the greatest amount of drinking were either in, or projected to the hippocampus and it was hypothesized that hippocampal after-discharges might be responsible for drinking. Macphail (1968) investigated the effect of carbachol stimulation on the electroencephalographic activity of the hippocampus, cortex, and amygdala. Carbachol-induced drinking occurred both during and in the absence of hippocampal slow waves and also failed to occur on a number of occasions when slow waves were present. Thus, hippocampal slow wave activity does not appear to be related to drinking. A second experiment showed that carbachol produces drinking through its central effects since intraperitoneal injections of carbachol did not produce drinking.

Since many of the sites from which a drinking response can be elicited are located near the midline, Routtenberg (1967) argued that carbachol may diffuse from the limbic system via the ventricles to other active sites near or within the ventricles which are responsible for drinking behavior. The ventricular diffusion hypothesis has also been supported by Baxter (1967). Carbachol elicited a similar emotional reaction whether it was placed in the hypothalamus, amygdala, or hippocampus of a cat whereas electrical stimulation of these structures resulted in differential emotional reactions. Since carbachol always produced a similar response regardless of the structure stimulated Baxter concluded that the carbachol may diffuse through the ventricular system to a specific locus that controls the response.

Mountford (1969), however, found that application of carbachol to the dorsal hippocampus resulted in a significant increase in drinking while placement of carbachol directly into the lateral ventricles had little effect. Myers and Cicero (1968) and Khavari, Heebink and Traupman (1968) injected various doses of carbachol directly into the lateral ventricles of rats and found no increase in drinking over normal baseline levels. The latter authors, however, reported that intraventricular atropine produced a reliable reduction in drinking by water deprived rats. The effects of intraventricular atropine on carbachol induced drinking was not investigated and it appears that ventricular diffusion does not play a major role in carbachol induced thirst.

The ventricular diffusion hypothesis has recently been revised. Simpson, Martin, and Routtenberg (1973) reported that cholinergic agents which are placed in the brain may be transported via the vascular system to the subfornical organ where activation of a drinking response is initiated. Direct application of carbachol to the subfornical organ resulted in a shorter latency and greater magnitude drinking responses than any other structure.

Stein (1963) differentiated between the central and periperal effects of atropine and scopolamine. Centrally active atropine and scopolamine reduced the water intake of deprived rats while their peripherally active analogues, atropine methyl nitrate and scopolamine methyl nitrate, had little effect on deprivation induced drinking.

De Wied (1966) reported similar results using hypertonic salt solution injections to increase extracellular osmotic pressure and produce drinking in rats. Both scopolamine and atropine reliably reduced water intake caused by hypertonic loads while peripherally active, atropine methyl nitrate, was less active in reducing water intake. These findings indicate that the action of the blocking agents must be central rather than peripheral. In fact, De Wied suggests that the reduction in the amount drunk following the "salt arousal of drinking" might be used as a general technique to classify centrally active anticholinergic agents.

Within the thirst circuit Singer and Montgomery (1970) investigated the functional relationship between the septal and amygdaloid nuclei. Carbachol stimulation of the lateral septal area caused sated rats to drink and this drinking was augmented by simultaneous stimulation of the amygdaloid cortical nucleus. Simultaneous anticholinergic stimulation of the amygdala abolished the effect of carbachol on the lateral septal nucleus and drinking decreased to control levels. Russell, Singer, Flanagan, Stone, and Russell (1968) demonstrated that application of carbachol to the amygdala increased drinking in rats deprived of water for three or eleven hours but did not increase the amount of water consumed by 23 hr. deprived rats nor did it initiate drinking in sated rats. Thus, differential lengths of deprivation may effect the potency of carbachol stimulation. After 23 hrs. of water deprivation the thirst circuit may be operating at full capacity and therefore the addition of carbachol may have little net effect on drinking.

The evidence presented above supports a circuit theory of thirst in which it is necessary for all components of the circuit to be functional in order for thirst and drinking to occur. Levitt and Fisher (1967), however, raised a serious objection to the circuit theory of thirst and questioned its generalization to explain natural thirst. Levitt and Fisher found that while atropine blocked cholinergically induced thirst it had little effect on natural thirst produced by water deprivation.

Stein and Levitt (1971) further investigated the effects of interrupting the thirst circuit by chemical blockers or radio frequency (RF) lesions. According to the circuit theory of thirst a RF lesion within this circuit should duplicate the blocking effect of atropine. However, drinking was depressed only when lesions were made in the lateral hypothalamus. Anterior thalamic and lateral septal lesions had little effect on carbachol induced drinking. Since RF lesions had little effect on drinking, the authors argued that atropine does not block cholinergically induced drinking by means of a temporary functional lesion. Atropine may, however, selectively affect cholinergically sensitive tissue at the site of injection whereas a RF lesion may nonspecifically destroy several overlapping systems, some of which may be opposite in action, and therefore no net change in behavior may appear following the lesion.

Strong evidence for the existence of a limbic neural circuit underlying drinking behavior has been presented by Buerger, Levitt, and Irwin (1973). Electrical multiple-unit recordings were made from the lateral

hypothalamus, lateral septal nucleus, and the caudate nucleus following unilateral carbachol stimulation. Large increases in neural firing were recorded at the cholinergically injected sites and at the contralateral noninjected sites in the lateral hypothalamus and lateral septal nucleus. Increases in multiple-unit activity were not recorded from the caudate nucleus which is not part of the diffuse thirst circuit. Thus, in sites where carbachol is effective in eliciting drinking it causes an increase in neural activity which is also evident in the contralateral homologous site. The time course of the increase in neural firing was also found to be similar to the time course of water ingestion. The increase in neural activity of the lateral septal area was of longer duration than the lateral hypothalamic increase. This difference in neural activity correlates with the greater amount of drinking which occurs following cholinergic stimulation of the septal area.

Preference behavior under chemical and natural thirst

Differences in preference behavior exist between animals which are naturally thirsty and those which are chemically induced drinkers. The thirst circuit hypothesis assumes that drinking induced by cholinergic stimulation has the same causal mechanism as natural thirst. It also assumes that the motivational characteristics of carbachol induced drinking are similar to those induced by deprivation in that rats under either condition will work to obtain water. However, differences in fluid preference behavior have been reported. Gandelman, Panksepp, and Trowill (1968) compared preference for a sucrose solution or water in

deprived and carbachol induced drinkers. In a two bottle preference test rats stimulated with carbachol in the medial septal area preferred a sucrose solution whereas the deprived group preferred water. This difference, however, was eliminated when only a single test fluid was present.

In an alcohol preference-aversion study, Cicero and Myers (1969) found that rats drank significantly more alcohol following water deprivation than following carbachol stimulation. Rats that received carbachol injections into various limbic structures rejected even normally preferred alcohol concentrations. The aversion to alcohol is in direct contrast to the water deprivation condition in which alcohol was preferred, indicating that natural and chemically induced thirst may not be qualitatively identical. It appears that palatability may play a greater role in chemically induced thirst or perhaps carbachol stimulation may result in a greater taste sensitivity.

Motivational properties of chemical and natural thirst

The problem is to determine whether the effects of artificial stimulation are the same as those produced by natural thirst. If the effects are the same, stimulation of the thirst circuit should serve to activate those behaviors which lead to water ingestion. Tenen and Miller (1964) showed that electrical stimulation of the lateral hypothalamus has motivational properties similar to natural thirst. Increased hours of deprivation as well as increased electric current intensity resulted in greater tolerance of quinine adulterated food. When deprivation and electrical stimulation were combined, a greater

concentration of quinine was tolerated than when either condition applied alone.

Coons, Levak, and Miller (1965) demonstrated that electrical stimulation of the lateral hypothalamus, which elicits eating, will also motivate the learning of a food-rewarded bar press response. This learned response was also observed to transfer to conditions of natural hunger.

Khavari and Russell (1966) compared the motivational properties of direct cholinergic stimulation of the lateral hypothalamus with those arising from water deprivation. In both straight alley and T-maze tasks cholinergic stimulation had properties similar to water deprivation in eliciting drinking and also in maintaining a response learned under water deprivation. Thus, it appears that the effects of cholinergic stimulation go beyond the initiation of consummatory responses and include motivational properties as well.

In a series of experiments Franklin and Quartermain (1970) compared the drive strength elicited by carbachol stimulation of the lateral preoptic area with that elicited by water deprivation. Twenty-three hours water deprivation produced the same amount of drinking as carbachol stimulation. VI bar pressing rate, however, was lower for the carbachol group, and the deprived rats also tolerated a stronger quinine solution than did the carbachol stimulated rats. Although the two groups would consume equal amounts of water if allowed free access to water, the motivational properties, as measured by VI bar pressing and quinine tolerance, indicate that natural thirst leads to greater motivation.

It is possible that deprivation induced thirst may result in stronger motivation because it is associated with a complex of stimuli such as, changes in the osmolarity of the body fluids and a reduction in blood volume, which do not accompany carbachol stimulation. Thus, deprivation may stimulate a variety of systems involved in water intake while carbachol activates only a single locus. This conclusion is supported by the findings of Fitzsimons and Oatley (1968) who demonstrated the additivity of thirst systems. Both intracellular dehydration produced by hypertonic injections and extracellular dehydration produced by hemorrhage lead to drinking. However when both stimuli were present as in natural thirst their effects were additive and a greater amount of drinking occurred.

Rolls, Jones, and Fallows (1972) compared the motivational properties of angiotensin and deprivation induced thirst. Angiotensin is not a cholinergic substance. Its precursor, renin, is produced by the kidneys in response to a reduction in blood volume. Renin is then converted to angiotensin which has been shown to elicit drinking when injected systemically or centrally into limbic sites (Levitt, 1971). Rolls et al. found that angiotensin caused rats to drink to satiation more quickly than water deprivation although there was no difference in total amount drunk. The 24-hr. deprived group also ingested significantly more quinine solution than did the angiotensin group. There was, however, no difference between groups on a progressive bar-pressing schedule. On this schedule a rat could obtain water by pressing a bar but each reward required an increased number of bar presses. This bar-

press data is inconsistent with the VI deficit in stimulated rats reported by Franklin and Quartermain (1970). Thus, it appears that the motivational effects of chemical stimulation may be similar to natural thirst and may also be task dependent.

Krikstone and Levitt (1970) investigated the interaction between level of water deprivation (9, 15, 23 hrs.) and conditions of chemical brain stimulation (unilateral carbachol, bilateral atropine, unilateral carbachol and contralateral atropine). Carbachol produced an increase in the amount drunk by all groups and the increment was the same across the three deprivation levels. Atropine reduced the amount drunk by all three groups. When carbachol and atropine were contralaterally injected the amount ingested was reduced to a level near the deprivation level. Since anticholinergic drugs completely inhibited drinking induced by chemical stimulation but only slightly inhibited natural thirst Krikstone and Levitt concluded that the neural mechanisms underlying natural thirst and chemically induced thirst may not be identical.

Blass and Chapman (1971) questioned the role of cholinergic mechanisms in thirst and suggested that acetylcholine may play only a minor role in drinking behavior. Atropine was found to be an effective blocker of extracellular thirst but not of cellular dehydration induced thirst. Atropine injections also had little effect on nonregulatory prandial drinking of desalivate rats. The authors argue that atropine is ineffective in blocking natural thirst because acetylcholine is only one of many neurohumors involved in drinking and that the potency of carbachol has resulted in undue emphasis on cholinergic mechanisms in

thirst.

Since central administration of atropine will block chemically induced thirst but not natural thirst and there are apparent differences in preference-aversion functions for alcohol and sucrose, Levitt (1971) concludes that the mechanism responsible for carbachol induced thirst may not be identical to natural thirst resulting from cellular dehydration and hypovolemia. Equivocal results have also been obtained in assessing the motivational properties of chemical and natural thirst using VI performance and quinine tolerance tests. The present experiment was designed as a further test of the motivational properties of chemical and natural thirst using a conditioned aversion paradigm. In this paradigm a rat consumes a substance and immediately following consumption is injected with a toxic substance such as lithium chloride (LiCl) which results in gastrointestinal upset. On the second presentation of this substance the rat will avoid it (Revusky and Garcia, 1970). Peters and Reich (1973) have shown that the aversion conditioning paradigm is sensitive to appetitive motivational differences induced by deprivation or ventromedial hypothalamic lesions. The present study investigated (1) the interaction between water deprivation and carbachol stimulation of the dorsal hippocampus and (2) the effect of deprivation and carbachol stimulation on the strength of a conditioned saccharin aversion. If carbachol stimulation results in an increase in water motivation it should enhance the drinking of deprived rats and lead to a passive avoidance deficit in the aversion conditioning paradigm.

METHOD

Subjects

The subjects were 51 male hooded rats of the Long-Evans strain approximately 100 days old at the outset. The rats were individually housed with constant illumination. All behavioral testing took place in the home cage.

Procedure

A single 21-gauge stainless steel cannula assembly was stereotaxically implanted and cemented to the skull under sodium pentobarbital (42mg/kg) anesthesia. The cannula was aimed at the dorsal hippocampus according to de Groot (1959) coordinates: AP= 3.0; H= 2.5; L= 2.5. A 4 day recovery period with free access to food and water followed surgery.

Preliminary screening procedures were used to determine which rats showed a positive drinking response to carbachol stimulation. The screening procedure consisted of a sham stimulation period followed by a carbachol stimulation period each lasting 1 hr. In the sham stimulation period the inner cannula was removed, cleaned, replaced and the amount of water consumed recorded. In the carbachol stimulation period that followed, crystalline carbachol was tapped (6-8 taps) into the inner cannula before insertion into the guide cannula. The amount of water consumed in the hour following stimulation was recorded. A rat that drank at least 10.0 ml. in the first screening session or at least 4.0 ml. on two successive screening sessions was classified as a carbachol induced drinker. Screening took place every third day until each operated rat

could be classified as a drinker or nondrinker. Rats that did not drink in response to carbachol stimulation served as operated controls. Following the screening procedure the carbachol drinkers and control rats were randomly assigned to one of three (6, 15, or 23½ hr.) water deprivation schedules. Surgery was performed on as many rats as necessary to obtain nine drinkers and eight control rats under each deprivation condition.

Following the screening procedure all rats were placed on their respective deprivation schedules for the remainder of the experiment. The first 10 days served as a period of adaptation to the drinking schedules. Water was available for 30 min. each day following the deprivation period and the amount consumed during this period served as the dependent measure. Food was available at all times except during this drinking period. Water was not returned to the 6 and 15 hr. deprivation groups until 2 hr. after the daily drinking session.

The carbachol groups received pretreatment with carbachol 5 min. prior to the drinking periods on days 11 and 14. The amount consumed on these days was an indication of the interaction between various levels of water deprivation and carbachol stimulation. Day 17 was the aversion conditioning day on which a 0.1% saccharin solution was present during the drinking period. Immediately following this session all rats were injected with a 2% body weight dose of .15M LiCl. Saccharin solution was then presented every third day to test the magnitude of the aversion in each group. Five test days were run with water present on the intervening days. Carbachol was placed into the dorsal hippocampus of the

carbachol groups on each test day.

At the conclusion of aversion testing all rats were given free access to food and water. After 2 days a final screening test was used to determine whether or not the carbachol drinkers maintained their positive response to carbachol stimulation throughout the experiment. This single screening test was identical to the initial screening procedure. Only the data from those rats that showed a positive drinking response (at least 4.0 ml.) to carbachol on this test were used in the analysis.

Histology

At the conclusion of behavioral testing the rats were sacrificed with an overdose of sodium pentobarbital and perfused with physiological saline followed by a 10% formalin solution. The brains were removed and frozen sections taken at 150μ. A photographic enlargement of each section was used to determine the exact locus of stimulation and the extent of neural damage caused by the carbachol or the cannula.

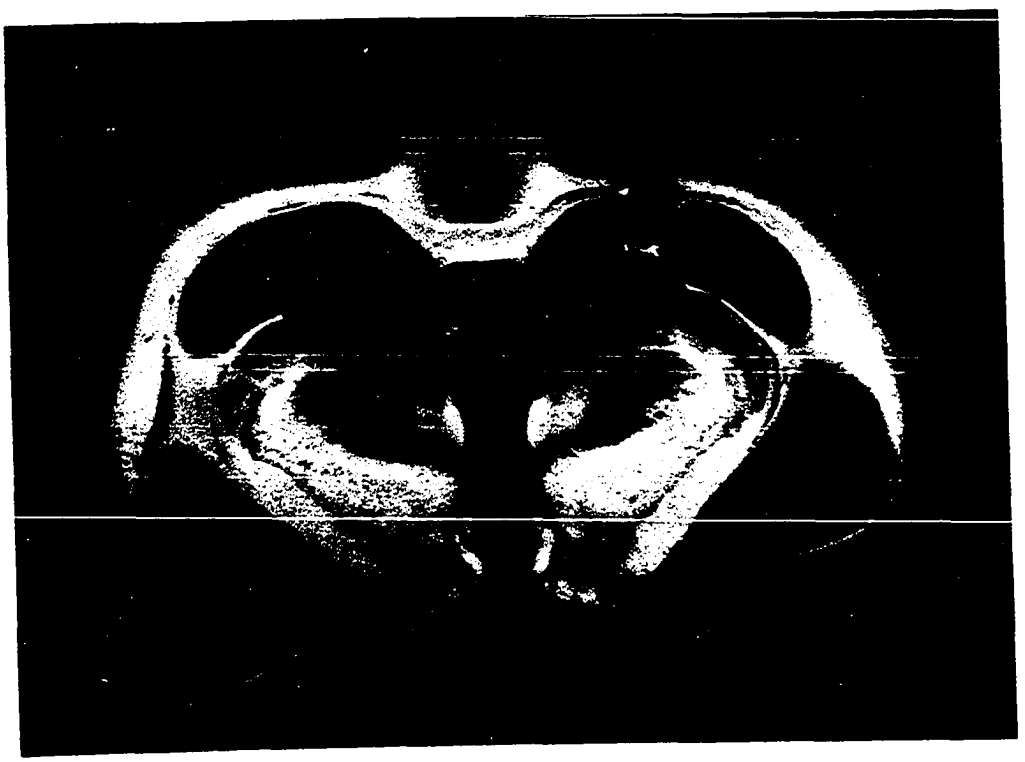
RESULTS

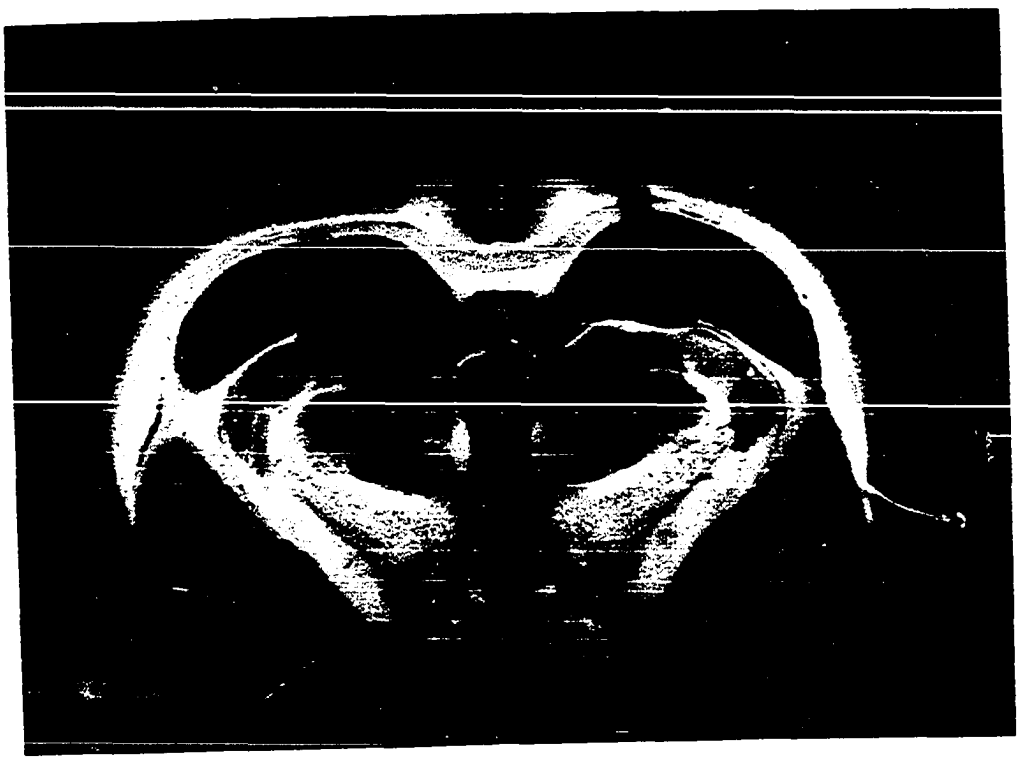
One rat died during the course of the experiment and the data from six other animals were subsequently discarded because drinking on the final screening session did not reach criterion and it was histologically determined that the locus of carbachol stimulation was ventral to the dorsal hippocampus. Examination of the brain sections of all rats which met the drinking criterion on the final screening test showed that the cannulas terminated in the dorsal hippocampus. The range of the positive placements according to de Groot (1959) coordinates was: AP= 1.8-4.4; H= 3.5-1.5; and L= 1.5-3.0. Representative brain sections of a carbachol drinker and non-drinker are shown in Figure 1. Eight rats remained in each of the control groups while there were six, eight, and six rats in the 6 hr., 15 hr., and 23½ hr. carbachol groups respectively.

Figure 2 shows the mean water intake at each level of deprivation for the carbachol and control groups following carbachol stimulation. A difference score was obtained between the sum of the amount consumed on the stimulation days, 11 and 14, and the amount consumed on the preceding days, 10 and 13. These difference scores were analyzed using analysis of variance. Water intake increased with hours of deprivation and carbachol stimulation caused an overall significant increase in water drinking ($F= 13.41$, $df= 1/38$, $p<.01$). The interaction between hours of deprivation and carbachol stimulation was not significant indicating that the increment in drinking resulting from carbachol stimulation was approximately equal across the three levels of deprivation.

Figure 3 shows the mean saccharin intake by each group on the aversion conditioning day and on the five aversion test days. Injections

Figure 1. Representative brain sections showing location and neural destruction caused by the cannula implant. Top photograph shows a positive implant site. The bottom photograph shows a negative site located ventral to the dorsal hippocampus.





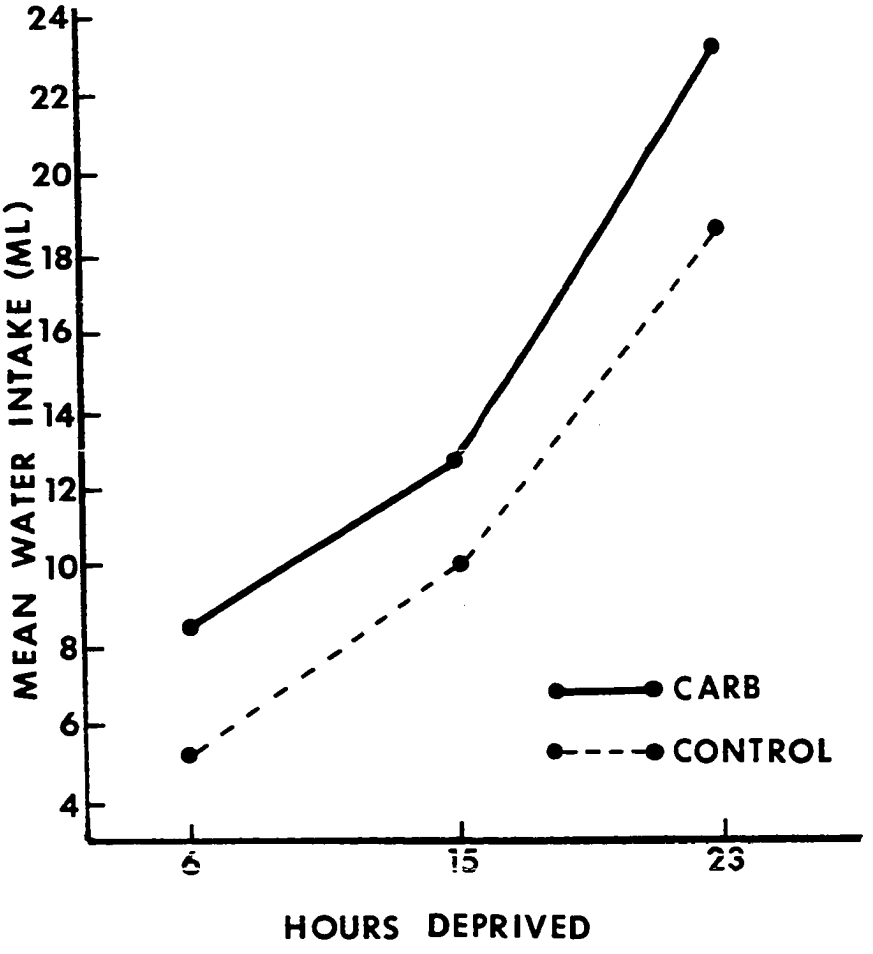


Figure 2. Mean water intake of the carbachol and control groups at each level of deprivation.

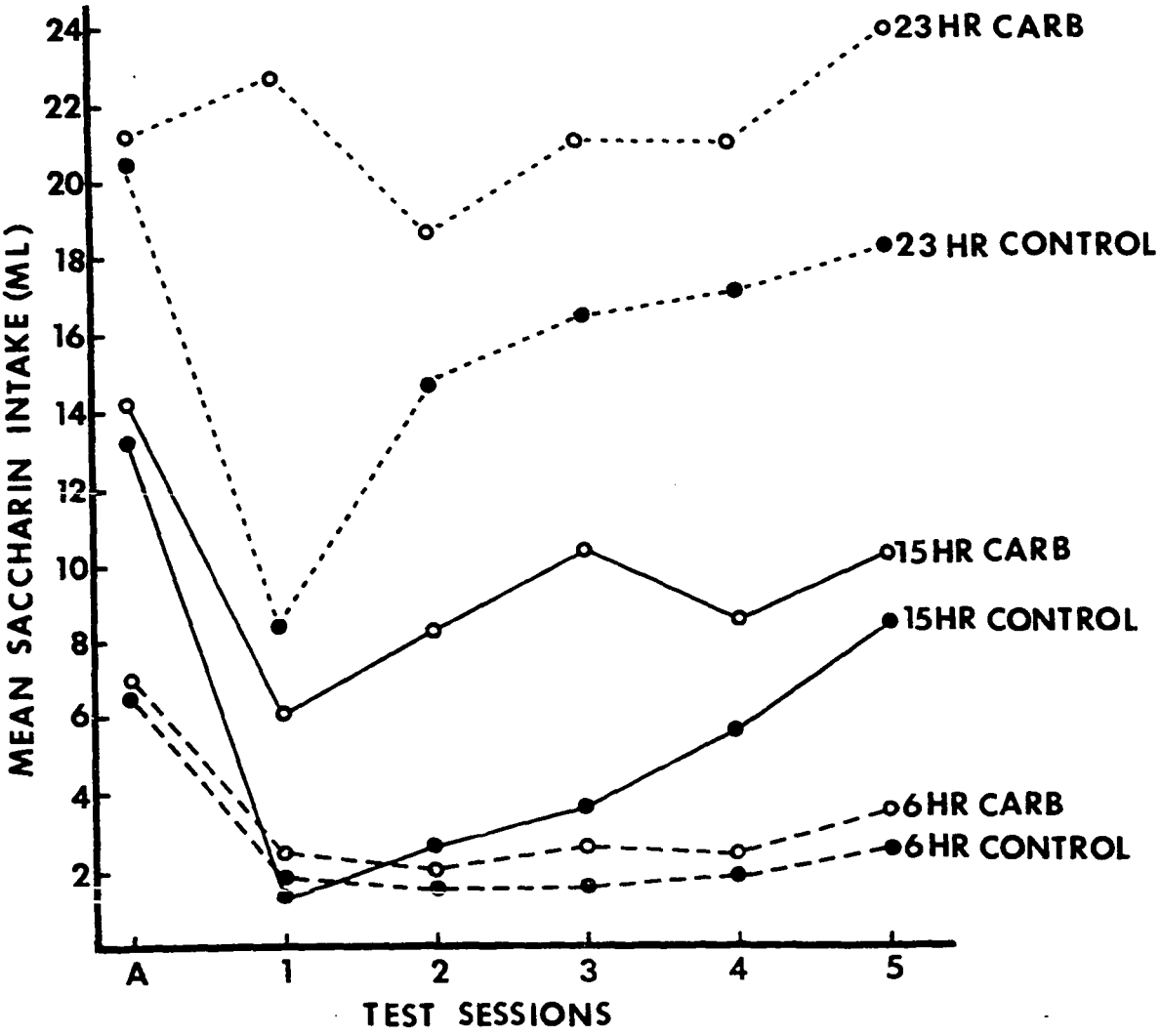


Figure 3. The mean saccharin intake by each group on the aversion conditioning day (A) and on the five aversion test days.

of LiCl caused a significant decrease in saccharin consumption on the first test day in all three deprivation control groups and in the 15 hr. carbachol group ($p<.05$). The decrease in the amount consumed by the 6 hr. carbachol group also approached significance ($p<.07$). The $23\frac{1}{2}$ hr. carbachol group, however, did not display an aversion and, in fact, a slight increase in saccharin consumption occurred on the first test day. As can be seen in Figure 3 within each level of deprivation the carbachol groups consumed a greater amount of saccharin. There was a significant overall carbachol effect across testing sessions ($F= 8.96$, $df= 1/38$, $p<.01$) as well as a significant deprivation effect ($F= 71.13$, $df= 2/38$, $p<.001$). There was no carbachol X deprivation interaction.

Subsequent *t*-tests between carbachol and control groups at the same level of deprivation showed a significant difference between the $23\frac{1}{2}$ hr. carbachol and control group on the first day of aversion testing. The carbachol group drank significantly more saccharin and did not show an aversion. All other similar between group comparisons failed to reach significance.

There was also a significant test day effect ($F= 21.46$, $df= 5/190$, $p<.001$). Referring to Figure 3 there was an increase in amount consumed across test days in each group. Comparing the first test day with the last test day showed a significant increase in saccharin intake across test sessions by the 15 hr. control group ($t= 3.79$, $df= 14$, $p<.01$), the 15 hr. carbachol group ($t= 2.90$, $df= 14$, $p<.05$) and the $23\frac{1}{2}$ hr. control group ($t= 5.38$, $df= 14$, $p<.001$). The $23\frac{1}{2}$ hr. carbachol did not display an aversion and there was no significant difference in saccharin intake

across test days. The 6 hr. groups did not show a significant increase in saccharin intake across test days. These comparisons show the effect of deprivation as well as the effect of deprivation plus carbachol stimulation on the duration of an aversion. It should be noted, however, that with the exception of the 23½ hr. carbachol group, the initial level of saccharin intake was always the greatest.

There were significant carbachol X test day ($F = 2.55$, $df = 5/190$, $p < .05$) and deprivation X test day ($F = 2.09$, $df = 10/190$, $p < .05$) interactions. Such interactions are expected since the decrease in saccharin consumption produced by aversion conditioning procedures affect the amount consumed following water deprivation and also following carbachol stimulation. The strength of the aversion also decreases across test days. The carbachol X deprivation X test day interaction was also significant ($F = 2.21$, $df = 10/190$, $p < .05$) indicating that the differences between groups at the three levels of deprivation were not the same.

Tables of raw data are given in Appendix A and Appendix B shows the source tables for the analyses which were performed.

DISCUSSION

There are three major findings that resulted from this study. First, carbachol stimulation of the dorsal hippocampus resulted in a significant increase in water intake by deprived rats. This result is in agreement with the findings of Grant and Jarrard (1968) and Mountford (1969) who reported that carbachol stimulation of the dorsal hippocampus resulted in increased water intake by sated rats. The present experiment extends these previous results to show that cholinergic stimulation of the dorsal hippocampus also leads to increased drinking by deprived rats.

The increase in water intake displayed by the 23½ hr. carbachol group is in direct contrast to the results reported by Russell et al. (1968). These authors found an interaction between cholinergic stimulation of the amygdala and hours of water deprivation. The greatest increase in drinking occurred following 3 hrs. of deprivation and no significant increase was evident following 23 hrs. deprivation. The authors concluded that 23 hrs. water deprivation may activate the thirst circuit to its fullest capacity and, therefore, the addition of carbachol would have no net effect on drinking. Since Russell et al. allowed their rats 1 hr. access to water on each test session, this discrepancy cannot be explained by possible ceiling effects imposed by a short duration test session and may be due to the different structures stimulated. In the present study there was no interaction between carbachol stimulation and hours of deprivation indicating that the thirst circuit can be modulated following 23½ hrs. water deprivation.

Krikstone and Levitt (1970) also reported the lack of interaction between carbachol stimulation and level of deprivation following

stimulation of the lateral hypothalamus, anterior thalamic region, and lateral septal nucleus. Animals that were both deprived and stimulated showed greater drinking than animals under either condition alone. Thus it appears that carbachol stimulation acts like water deprivation in that it increases drinking and it appears to specifically add a constant factor to whatever level of thirst is present.

The second major finding, that the duration of a conditioned aversion is a function of deprivation level, confirms the earlier findings of Peters and Reich (1973). The present experiment extends the previous findings based on hunger research to include thirst. The animals under the lowest level of deprivation did not increase the amount of saccharin solution consumed across the test sessions whereas control rats in the 15 and 23½ hr. groups did increase saccharin intake. The level of deprivation affects the absolute amount consumed as well as the rate at which the aversion dissipates. Since significant differences occur between groups differing in deprivation level by as little as 8 or 9 hrs., it appears that the aversion conditioning paradigm serves as a sensitive measure of motivation resulting from hunger and thirst.

The third major result was that carbachol stimulation of the dorsal hippocampus resulted in increased thirst motivation as indicated by the significant carbachol effect across aversion testing sessions. As shown in Figure 2, without exception, within each level of deprivation the carbachol group shows a greater mean saccharin intake. Since the amount consumed also increased with hours of deprivation it appears that carbachol stimulation produces behavior similar to more thirsty animals.

The effect of carbachol stimulation was most apparent in the 23½ hr. carbachol group which did not display an aversion to saccharin on the first test session. Thus it appears that carbachol adds to natural thirst in a manner similar to increased hours of deprivation and the present results are consistent with those reported by Grossman (1962a,b) and Khavari and Russell (1966).

Recently Johnson and Fisher (1973a) have questioned the generality of the preference differences reported by Gandelman et al. (1968) who demonstrated that deprived animals prefer water to sucrose solutions while cholinergically stimulated animals prefer sucrose to water. Johnson and Fisher found a preference for water by both the stimulated and deprived groups. Both groups showed nearly identical drinking patterns in a two bottle choice situation. The animals begin by ingesting a large quantity of sucrose and drank water only late in the test session. Sucrose consumption did not increase with deprivation but water intake did increase with hours of deprivation. Thus, the amount of water intake is a function of the absolute water deficit of the animal as well as a function of the duration of the test period. If the test session is very brief an apparent sucrose preference would result.

In a second experiment designed to test quinine tolerance under cholinergic and natural thirst Johnson and Fisher (1973b) found that carbachol induced drinkers tolerated significantly lower concentrations of quinine than deprived animals when tested 10 min. after stimulation. There was, however, no significant difference between groups when 25 min. had elapsed between stimulation and testing. Thus, cholinergic thirst

may increase for a period of time and converge with natural thirst. Based on the above two studies the authors conclude that there is no basic qualitative difference between chemical and natural thirst.

The present experiment demonstrated that there exists within the hippocampus a cholinergically coded thirst circuit. Carbachol stimulation of this circuit leads to increased water intake as well as increased thirst motivation. However, it is impossible to equate the activity of this system with natural thirst since Levitt and Fisher (1967) have shown that anticholinergic agents will block cholinergically induced drinking but will not block deprivation induced drinking. Therefore, the mechanisms underlying natural thirst and chemical thirst may not be qualitatively identical. The lack of interaction between carbachol stimulation and hours of deprivation in the present study may also suggest that the two systems are separate. Since equivalent increments in drinking resulted across levels of deprivation, carbachol may be activating an additional system which is capable of initiating and modulating drinking but which is not necessarily responsible for natural thirst.

In summary the present experiment has shown that carbachol stimulation of the dorsal hippocampus results in increased water intake as well as increased saccharin intake in a conditioned aversion paradigm. Thus, cholinergic stimulation of the thirst circuit results in not only increased drinking but also in increased thirst motivation. It is questionable, however, whether the neuroanatomical systems underlying cholinergic and natural thirst are identical.

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ACKNOWLEDGEMENTS

I would like to express my appreciation to my advisor, Dr. Ronald H. Peters, for his assistance in the design of this study and for his criticisms of the manuscript. I also thank him for the interest and guidance he has shown and for the research techniques he has taught me. I would also like to thank the members of my graduate committee: Dr. Leroy Wolins, for his statistical assistance, Dr. David C. Edwards, Dr. Gary Phye, and Dr. Benton Buttrey for their criticisms and the interest they have shown in this dissertation.

I would also like to express my appreciation and thanks to my wife, Kitty, not only for the typing of this manuscript but also for the sacrifices she has made and the support and encouragement she has given me while at Iowa State University.

APPENDIX A

Table 1a. Amount of fluid consumed by the 6 Hr. deprivation control group across the 32 day testing period. Amount recorded in ml.

testing days

Animal	1	2	3	4	5	6	7	8	9	10	11
176	11	7	6	5	4	5	9	6	8	9	9
177	6	3	5	4	1	7	1	6	7	6	4
186	1	0	0	1	1	1	2	1	2	4	2
189	4	2	3	3	3	9	3	2	2	4	4
192	3	4	1	3	6	10	6	9	7	9	9
200	9	6	4	3	3	4	6	4	5	4	3
210	5	2	5	5	6	5	5	8	11	11	7
218	11	4	4	6	3	5	4	3	0	4	1

Animal	12	13	14	15	16	17	18	19	20	21	22
176	7	7	8	8	7	6	6	6	1	3	3
177	7	1	9	9	7	8	5	9	2	6	3
186	2	2	0	1	1	2	3	4	1	4	4
189	3	5	3	2	4	3	4	3	2	5	4
192	12	10	9	12	12	12	8	11	3	4	6
200	3	5	9	3	3	2	5	7	3	3	5
210	7	7	7	6	5	8	4	4	2	1	2
218	4	1	1	1	5	11	5	2	1	6	5

Animal	23	24	25	26	27	28	29	30	31	32
176	3	6	7	3	6	5	1	5	7	5
177	2	7	12	1	6	6	2	8	5	2
186	0	1	5	1	1	3	2	4	2	2
189	2	6	5	2	4	4	5	5	3	6
192	1	6	7	1	5	4	1	5	4	0
200	2	3	7	1	7	8	2	9	6	2
210	1	2	4	2	1	5	1	6	7	2
218	2	5	7	1	6	6	1	6	7	1

Table 1b. Amount of fluid consumed by the 6 Hr. carbachol group across the 32 day testing period. The amount consumed on day 35 represents the final screening test. Amount recorded in ml.

testing days

Animal	1	2	3	4	5	6	7	8	9	10	11
175	8	11	6	4	7	9	7	6	4	5	9
179	5	5	7	6	6	6	8	5	7	6	8
190	4	4	5	5	4	6	6	6	5	6	15
194	6	6	6	6	6	3	4	4	6	8	8
207	3	1	3	1	3	2	5	4	6	5	7
217	7	5	6	5	5	4	4	1	3	1	5

Animal	12	13	14	15	16	17	18	19	20	21	22
175	4	1	9	4	6	8	6	5	2	5	5
179	10	6	8	6	9	6	9	8	3	10	7
190	9	7	13	6	7	8	11	6	3	5	6
194	6	6	8	10	7	8	7	8	2	9	7
207	8	4	5	5	9	6	6	5	1	7	4
217	0	1	8	2	1	5	1	1	3	4	2

Animal	23	24	25	26	27	28	29	30	31	32	35
175	2	5	5	3	5	6	1	5	7	7	4
179	1	10	8	1	7	6	2	13	5	2	4
190	1	5	5	5	2	2	5	5	4	4	4
194	3	8	8	1	7	5	2	8	5	1	6
207	1	5	1	2	6	1	2	6	1	2	18
217	4	2	2	3	3	0	2	2	2	5	6

Table 1c. Amount of fluid consumed by the 15 Hr. deprivation control group across the 32 day testing period. Amount recorded in ml.

testing days

Animal	1	2	3	4	5	6	7	8	9	10	11
181	15	12	10	10	11	11	6	9	12	12	11
184	7	5	5	4	5	4	6	6	5	6	8
185	10	8	5	9	8	7	6	10	11	10	9
187	10	17	13	13	12	13	15	10	15	15	16
196	11	11	13	13	13	11	14	14	11	12	11
201	8	7	7	6	5	7	9	5	10	9	5
212	12	8	8	6	9	8	8	8	6	11	9
221	11	12	16	13	16	16	21	16	15	15	11

Animal	12	13	14	15	16	17	18	19	20	21	22
181	10	10	9	12	11	14	10	10	2	10	13
184	7	6	5	8	8	8	7	6	2	5	5
185	11	12	14	11	11	13	11	13	1	9	10
187	13	11	15	11	12	17	8	11	1	8	11
196	10	8	3	11	6	15	6	6	2	7	7
201	10	8	9	11	7	10	9	5	1	11	6
212	13	10	11	11	10	12	8	9	1	11	11
221	13	15	15	13	15	17	13	12	2	14	16

Animal	23	24	25	26	27	28	29	30	31	32
181	6	10	8	7	8	8	7	7	9	9
184	3	8	4	2	7	6	3	5	7	3
185	2	16	12	2	14	10	2	10	9	2
187	2	9	12	6	10	18	10	11	13	15
196	3	8	9	5	9	8	10	11	12	14
201	1	11	8	4	9	9	8	10	9	11
212	1	6	8	1	7	7	2	13	12	5
221	2	16	13	2	13	14	3	18	11	8

Table 1d. Amount of fluid consumed by the 15 Hr. carbachol group across the 32 day testing period. The amount consumed on day 35 represents the final screening test. Amount recorded in ml.

testing days

Animal	1	2	3	4	5	6	7	8	9	10	11
178	7	7	7	11	6	6	9	10	14	9	17
180	7	7	8	7	8	8	7	8	7	7	9
195	12	14	12	9	10	11	10	8	12	16	11
197	14	18	12	10	14	17	16	17	5	16	19
205	5	8	8	7	15	7	6	7	3	3	8
215	14	11	14	12	12	11	11	14	14	11	13
219	1	9	3	5	6	6	7	9	9	9	10
224	6	3	9	8	7	7	6	6	6	7	7

Animal	12	13	14	15	16	17	18	19	20	21	22
178	14	16	18	22	20	20	13	13	1	16	13
180	7	8	11	8	7	10	9	8	1	9	9
195	8	11	12	11	10	13	11	10	11	14	5
197	22	16	13	19	18	21	16	18	6	19	19
205	8	9	27	14	16	14	14	12	15	15	13
215	10	8	13	9	5	12	9	8	1	5	6
219	15	13	9	14	15	15	11	10	11	14	14
224	8	7	7	9	11	9	7	7	2	13	10

Animal	23	24	25	26	27	28	29	30	31	32	35
178	1	9	12	2	15	9	2	12	10	2	8
180	1	10	8	1	14	9	3	10	9	5	7
195	5	8	8	11	8	9	11	13	9	14	16
197	16	18	18	16	14	16	20	16	17	19	6
205	30	12	9	31	12	11	15	9	5	26	22
215	1	7	7	4	12	12	4	14	13	3	6
219	9	12	9	10	13	10	9	13	9	12	4
224	3	9	11	7	13	5	5	12	20	9	5

Table 1e. Amount of fluid consumed by the 23½ Hr. deprivation control group across the 32 day testing period. Amount recorded in ml.

testing days

Animal	1	2	3	4	5	6	7	8	9	10	11
183	12	11	13	14	5	15	15	14	14	16	17
188	12	13	14	13	13	5	14	15	18	15	19
191	7	13	12	14	13	15	13	16	14	16	18
199	15	17	15	19	17	18	18	18	17	18	19
203	15	16	17	13	17	12	18	17	15	17	16
211	14	16	15	14	16	17	19	16	19	15	18
216	14	14	19	20	22	22	20	21	23	25	23
225	10	10	12	11	14	18	19	15	20	20	18

Animal	12	13	14	15	16	17	18	19	20	21	22
183	18	16	17	18	16	18	13	13	9	16	20
188	18	18	17	18	19	21	16	19	10	23	24
191	16	15	17	16	18	20	15	15	15	17	17
199	18	18	19	17	19	18	13	15	3	19	17
203	19	16	17	16	19	20	15	18	10	18	19
211	21	19	18	18	18	20	14	15	5	9	16
216	20	25	23	22	22	24	13	18	12	22	21
225	21	21	22	21	22	23	20	20	4	20	19

Animal	23	24	25	26	27	28	29	30	31	32
183	18	18	17	18	18	18	18	16	16	18
188	19	23	22	23	24	23	24	23	22	24
191	16	17	15	18	16	17	20	14	17	18
199	8	17	16	5	9	15	13	15	14	13
203	19	16	20	18	21	19	21	20	16	16
211	13	18	18	14	20	19	14	18	17	19
216	13	22	20	21	21	19	17	17	18	19
225	12	18	24	18	21	8	11	21	22	19

Table 1f. Amount of fluid consumed by the 23½ Hr. carbachol group across the 32 day testing period. The amount consumed on day 35 represents the final screening test. Amount recorded in ml.

testing days

Animal	1	2	3	4	5	6	7	8	9	10	11
182	17	17	21	19	21	21	20	23	24	22	28
193	8	20	20	24	19	23	22	20	20	18	27
202	14	16	22	19	10	19	20	16	19	19	28
206	9	14	16	18	17	20	22	20	19	21	17
222	15	11	18	15	16	16	17	14	17	17	26
223	12	12	14	15	13	14	17	18	15	18	17

Animal	12	13	14	15	16	17	18	19	20	21	22
182	24	27	31	27	29	22	20	25	15	27	27
193	23	23	29	22	22	24	21	24	26	23	22
202	14	18	21	22	18	21	14	19	37	12	19
206	25	22	17	20	22	23	13	19	25	23	23
222	17	17	17	19	17	16	10	16	20	22	20
223	21	19	21	23	19	22	14	16	14	22	21

Animal	23	24	25	26	27	28	29	30	31	32	35
182	35	33	29	29	27	26	27	31	30	31	12
193	21	23	23	26	23	23	23	23	25	28	8
202	12	19	23	11	24	20	11	22	20	12	5
206	19	22	24	16	25	28	21	26	24	23	15
222	12	23	22	28	22	19	28	21	17	25	10
223	14	27	23	16	21	23	16	22	22	27	10

APPENDIX B

Table 2. Source table for analysis of difference scores used to determine effect of carbachol on water intake.

SOURCE	SS	DF	MS	F
Carbachol	470.05	1	470.05	13.41***
Deprivation	32.54	2	16.27	1.0
Carb X Dep	15.93	2	7.96	1.0
Error	1832.18	38	35.05	
		43		

***Significant at .001 level.

Table 3. Source table for the analysis of aversion test days.

SOURCE	SS	DF	MS	F
Carbachol	666.18	1	666.18	8.96**
Deprivation	10573.71	2	5286.85	71.13***
Carb X Dep	347.53	2	173.76	2.34
S/Carb X Dep	2824.55	38	74.33	5.62***
Days	1401.06	5	280.21	21.46***
Carb X day	166.52	5	33.30	2.55*
Dep X Day	272.81	10	27.28	2.09*
Carb X Dep X Day	288.16	10	28.82	2.21
S Day/Carb X Dep	2480.28	190	13.05	
		263		

*Significant at .05 level.
**Significant at .01 level.
***Significant at .001 level.